The Ichthyogram

April 1994 Volume 5 Issue 1

AQUACULTURE BILL PASSES UTAH LEGISLATURE

The Utah Legislature recently passed HB262, a bill dealing with private aquaculture. The bill moves authority for private aquaculture and fee fishing operations from the state Division of Wildlife Resources (DWR) to the Department of Agriculture (DAG). State hatcheries, private ponds and institutional aquaculture will continue to be regulated by the Division of Wildlife. The issues of fish health are expected to be the major topics for change under this legislation.

The bill calls for the creation of two boards. The **advisory board** will be made up of eight members and will represent private aquaculture, sport fishing interests, DAG and DWR. This board will give advice and make recommendations to a **policy board** made up of equal numbers of DAG and DWR appointees. The state veterinarian will serve as chairman of this board and the chief fish pathologist from DWR is also designated as a member. The bill states that current fish disease policies will be maintained until supplemental rules and regulations from DAG can be written.

The bill was opposed by several sportsmen's groups who remain unconvinced about the role of DAG in protecting aquatic wildlife. Private aquaculturists stressed their need for an agency that had the resources to provide service and promote their industry. The issue of providing fish health inspections was complicated by the state legislature earlier slashing general funding for DWR earmarked for private aquaculture inspections. A supplemental appropriation of $50,000 was added to this bill to fund DWR inspection services till July 1, 1994 when the transfer becomes official.

Speaking at the Bonneville AFS meeting, assistant state veterinarian Dr. Norm Erikson said that DAG has no preconceived plans on how it will administer the program and its first priority will be to hire qualified individuals. The bill requires that a certified fish health inspector conduct health inspections.

"If you like laws and sausages, you should never watch either one being made."
--Otto von Bismarck

inside...

Production of Sterile Trout Using Tetraploid Broodstock: Refining the Pressure Treatment Technique

Sterile fish can be a useful tool for fisheries managers and aquaculturists. Sterile grass carp, *Ctenopharyngodon idella*, have been used for aquatic weed control in areas where natural reproduction of the grass carp is undesirable. The potential superior growth of sterile fish and higher dress-out weights are also desirable attributes. Concerns for the genetic integrity of native fishes are increasing the need for sterile fish in stocking and commercial aquaculture programs. In Utah, there was a need for sterile rainbow trout (*Oncorhynchus mykiss*) to maintain the genetic purity of native subspecies of cutthroat trout (*O. clarki*) in waters where both occurred. The high fishing pressure and greater susceptibility of cutthroat trout to angler harvest required that rainbow trout be stocked in the same waters to sustain both the cutthroat trout population and recreational fishing.

Sterile fish can be produced by interspecie hybrids or by manipulating the number of chromosomes to create triploid individuals. Triploidy can be induced by shocking the fish eggs during meiosis, retaining the chromosomes of the second polar body. Alternatively, triploids may be created by an interploid cross, i.e., crossing tetraploids and normal diploids. This is the strategy being pursued by this study, since the percentage of triploidy derived from an interploid cross is reportedly more consistent: 93 and 99.5% in two trials by Chourrout et al. (Theor. Appl. Gen. 72:193), 95% triploid and pentaploid by Chourrout and Nakayama (Theor. Appl. Gen. 74:687), and 100% in a 60 fish sample tested by Myers and Hershberger (Aquaculture 96:97). Relative to heat-shock derived triploids, interploid triploids also had better survival to the fry stage.

To produce the tetraploid brood stock necessary for interploid crosses, shocking the embryos at 65% of the time to the first cleavage stage has proven to be a reliable technique. Researchers have further refined the technique by identifying the pressures (8,000 to 9,000 psi) required to optimize the percent tetraploidy.

This study was designed to continue improving the methodology for creating tetraploids while developing a tetraploid brood stock for production of sterile rainbow trout. This investigation evaluated the effect of type of fluid in a hydrostatic pressure chamber (HPC) upon survival and percent tetraploidy of pressure treated eggs.

Three types of hydrostatic fluid in the HPC were evaluated in the experiment: hatchery spring water, commercial sperm diluent solution or glycerin (absolute glycerol). Three separate pools of eggs (about twenty females and fifteen males) were fertilized at 30 min intervals to allow enough time for pressure treating each replicate. The pressure in the chamber was brought up to 9000 psi over a 30 sec interval by means of a 20 ton hydraulic press. The pressure of the chamber was maintained for 4 min, then depressurized over a 30 sec interval. Control eggs were poured into the chamber and left for five min, but no pressure was applied.

For each replicate, records of egg mortality, egg "eye-up", hatching success, and crippling rate were kept (Table 1). The data indicated that glycerol was a poor fluid to use in the chamber. Most of these embryos were lost during the first 24 h. Water and sperm diluent were comparable

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in their performance, so water is recommended for future pressure treatments.

When the fish were large enough to collect blood from, tetraploidy was determined by examination of blood smears, measuring the nuclear axes of red blood cells. Blood was also analyzed by flow cytometry to determine tetraploidy.

The results of the tetraploid assessment by flow cytometry indicated that 29 of 99 samples were tetraploid. The breakdown by treatment is in Table 2. Examination of blood smears corroborated all but four of these. This method detected 16 additional fish as tetraploids that were not identified by flow cytometry. Generally, flow cytometry is considered the more accurate of the two techniques.

Currently, there are about 16 tetraploid brood stock remaining of the roughly 9,000 eggs (0.18% survival) that were pressure treated. Some losses occurred after blood collection, due to the small size (about 75 to 100 mm, 1 yr P.F.) of some the tetraploids which did not grow well at the cold temperatures (9° C).

Fluid treatment	24 h P.F. egg survival (%)	Eye-up (%)	Hatch (%)	Survival after cripples removed (%)
control (water)	98.8	82.8	81.0	80.5
glycerol	23.2	11.0	5.4	4.3
sperm diluent	90.6	46.9	25.4	20.4
water	98.5	52.9	27.7	21.2

Table 1. Comparison of percent survival of rainbow trout to four stages of development after pressure treatment in a hydrostatic pressure chamber using three types of fluid.

Fluid Treatment	Number of Diploid	Number of Tetraploid
water	23	20
sperm diluent	43	7
glycerol	0	0

Table 2. Comparison of the number of tetraploids determined by flow cytometry created using 3 different fluids.

Eric Wagner

Video Observations of Rainbow Trout Behavior: Effects of Demand Feeders and Different Densities

. . demand-fed fish may retain more of their natural fear (wariness) of predators which may translate to better survival after stocking in the wild.

Demand feeders represent an important option for fish hatchery management. Fish can feed themselves at their own pace over a 24 hr period which allows them to grow at their greatest potential. In addition to improved growth, advantages to food conversion, water quality, and reduced labor have been reported. Some research has suggested that feeding to satiation alleviates the problem of aggression and fin-nipping in trout reared at conventional hatchery densities. Demand feeders represent an easy and practical way to feed fish to satiety, with little wastage of feed. Demand feeders also eliminate the "feeding frenzy" which results in wasted energy and drastic oxygen depletion following each feeding. Some researchers have also reported that demand feeders reduced size variation of fish probably due to a lack of competition for food. In this experiment, the hypothesis that feeding to satiation with demand feeders may alleviate aggression and fin-nipping in the hatchery environment was tested.

In the first experiment, 100 rainbow trout (13.6/lb) of the Sand Creek strain were stocked into each of two parallel raceways. The numerical density of fish was 3.5 fish/ft^3 and the initial loading density was 0.258 lbs./ft^3. This loading density was about 50% below the lowest rearing densities used in the Utah State Hatchery system (unpubl. data). In the second experiment, 50 fish were culled from each raceway after 8 wks so that half the density remained. A homemade demand feeder was made from a 5 gal white plastic bucket and was placed permanently in one raceway and the fish were fed *ad libitum*. In the adjoining raceway, the fish were hand-fed (0.10 lbs./day/100 fish) three times a day at 0800, 1130, and 1630 for five days a week (Mon-Fri). Both raceways received identical diets in the form of 5/32 inch sinking dry pellets. A small videocamera (Panasonic Palmcorder) was placed in an empty 5-gal aquaria and held (by a metal brace) sub-surface to permit underwater viewing without the presence of the cameraman required.

During the 10 min acclimation period after turning on the camera, the number of surface rises for natural prey was recorded and used as an indicator of return to normal behavior. The high density hand-fed fish had a significantly greater rate of rises than in the demand-fed fish (Figure 1A). This result suggests that the hand-fed fish were more habituated to human presence because of daily feedings. Alternately, demand-fed fish may retain more of their natural fear (wariness) of predators which may translate to better survival after stocking in the wild. The same trend was also apparent in the low density groups.

During the following 21-min observation period, there were no differences in the rising rate between hand-fed and demand-fed fish at either density (Figure 1B). This result was surprising since one might expect that trout fed *ad*

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libitum would not make an effort to rise for small insects.

Among the demand-fed fish, examined the number of demand feeder hits was also examined. The high density fish had 0.55 feeding bouts/min and the low density fish had 0.12 feeding bouts/min. On the basis of numbers of fish present, one would expect the low density group to have a higher feeding rate (about one half of the high density fish). This inconsistency suggests that more accidental bumping of the demand feeder rod may be occurring at the higher density, although the effects of social facilitation on feeding should not be ignored. Whatever the reason, further experiments on growth rates with demand feeders and different rearing densities are warranted by this result.

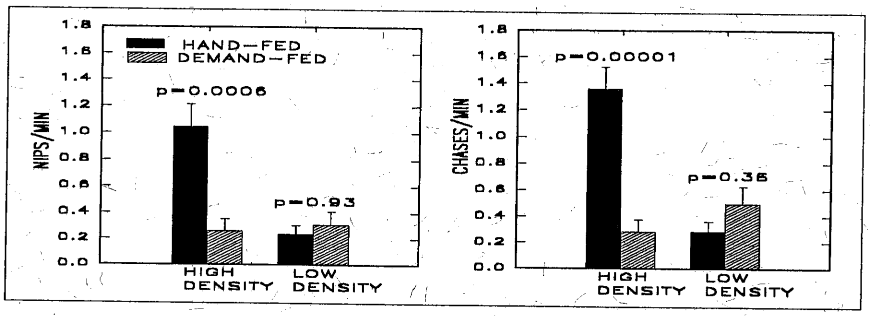

Figure 1. Surface rising rates of rainbow trout to natural prey during the 10-min acclimation period (left) and the following 21 min observation period (right). Bar height represent the mean and error indicates the SE. Bar height represents mean rate with SE indicated by reerror bars. P-levels refer to results of Mann-Whitney U-tests testing for differences between hand-fed and demand-fed groups.

Nipping and chasing are the predominant components of aggressive behavior in the rainbow trout (1). During the 21-min observation periods, the high density hand-fed fish showed significantly greater nipping and chasing rates (5-fold) than demand-fed fish (Figure 2). These differences confirm earlier observations (3,5) that feeding to satiety reduced aggression in trout. Conversely, food deprivation

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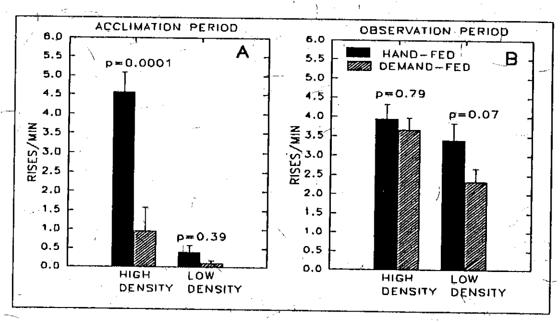


Figure 2. Nipping and chasing rates of rainbow trout during 21-min observations. Bar height represent mean rate with SE indicated by reerror batrs. P-levels refer to results of Mann-Whitney U-tests testing for differences between hand-fed and demand-fed groups.

Brake Trout: the Saga Continues

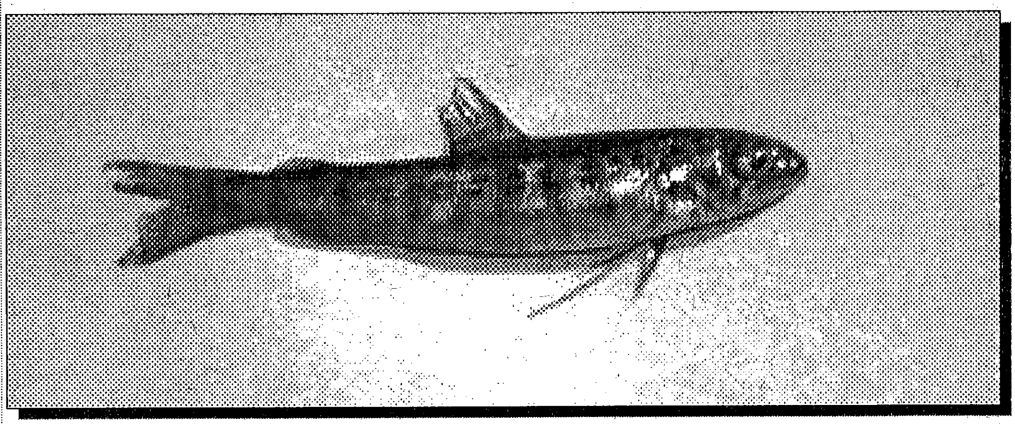
As reported in the last issue, the brake trout project is being conducted at the Fisheries Experiment Station. The "brake" is a cross between a brown trout female and lake trout male. (*The report of the cross from the previous article was incorrect. Editor)* The disease resistance of lake trout to whirling disease is an attribute that would be desirable in a stream-adapted salmonid such as the brown trout. The brown trout is reportedly resistant to whirling disease, although infected fish sampled from the Fremont River in October 1992 indicate that significant deformities occur in this species. In October 1993, brown trout eggs were taken from the Egan hatchery and then fertilized with lake trout from Fish Lake. The fertilized eggs were heat shocked to induce triploidy, a strategy which has improved survival of other inter-specie crosses.

About 56,000 eggs were received at the Fisheries Experiment Station on 12/22/93. A total of 33,163 dead eggs were picked off the day they were received. After hatching, sac fry were screened and 6,059 more dead eggs taken. Total hatch was 30.03% and the percentage of total cripples was 3.19%. There were about 15,000 fry on feed by 1/24/94. An inventory was taken on 3/25/94 to get an accurate count. Much to our dismay, there were only 3012 fish, far from the original inventory. Cannibalism remains a strong possibility, but an error in the previous counts is also a n explanation.

There are some differences in their culture characteristics to date. For example, the brake trout will not switch to a bigger size feed as fast as rainbow and cutthroat trout (still on #1 crumble feed at 377 fish/lb). Number 2 feed sinks before the fish have a chance to eat it. It appears that they eat very little food on the bottom of the troughs, so any food that gets to the bottom is wasted. The daily growth rate to date has been 0.018 inches per day at 55°F.

Some of the fish will eventually be put into live cages with rainbow trout to determine relative susceptibility or resistance to whirling disease. Meanwhile, the brake trout will continue to be monitored closely here at the station, and more information shared in future issues of the *Ichthyogram.*

Doug Routledge



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increased levels of aggression in juvenile Atlantic salmon (4).

In the low density fish, there was no trend for elevated aggression levels in the hand-fed group (Figure 2), with both groups similar to results for the high density demand-fed fish. Previous investigators (2) noted that high densities caused an increase in aggression levels of rainbow trout. The results of this study then indicate that demand-feeding can help alleviate aggression levels induced by higher densities.

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Thomas Bosakowski
(Editors note - Tom Bosakowski was "bumped"as part of the aftershock of the reduction in force process for the Division of Wildlife Resources. Tom has left Utah for Seattle, where he has accepted temporary employment with an environmental consulting firm. Goof luck, Tom)

Whirling Disease Established in Wild Rainbow Trout on Blacksmith Fork?

Acting on reports from local fishermen, biologists from FES and the DWR's Northern region recently electroshocked a feeder creek emptying into the Blacksmith Fork river in Cache county of northern Utah. Samples for whirling disease testing were obtained from 15 rainbows and rainbow-cutthroat hybrids which appeared to be wild fish, along with 59 brown trout. This section of stream was previously sampled in February 1993. At that time infected rainbow trout were discovered at a site immediately below a bridge, while cutthroat and brown trout were free of the parasite. These results led biologists to wonder if infected rainbow trout had been stocked (illegally) into the stream, as had been demonstrated in the nearby Little Bear River.

The results of the more recent testing showed that the wild rainbow and hybrids had moderate numbers of *Myxobolus cerebralis* spores. No young-of-the-year rainbow age classes were found. One rainbow showed moderate cranial deformities. In contrast, none of the brown trout were infected.

The evidence suggests that the parasite has become established among the more susceptible wild fish in a relatively short period of time. Consistent with other reports, brown trout appear to be relatively resistant at this time. Further testing and a possible reevaluation of fisheries management strategies for this stream are being planned.
Chris Wilson

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